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STUDIES OF THE PROTHORACICOTROPIC HORMONE IN THE TOBACCO HORNWORM, *MANDUCA SEXTA*

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Kopeć (1917, 1922) first demonstrated that mature larvae of the gypsy moth, *Lymantria dispar,* were unable to undergo metamorphosis when deprived of their brains. Additional experiments led him to conclude that a brain hormone stimulated metamorphosis. In the 1940's this conclusion became established as part of the classical theory of insect development and metamorphosis as a result of similar observations including those of Caspari and Plagge (1935), Kühn and Piepho (1936), Plagge (1938), Wigglesworth (1940), Schmeider (1942), and Williams (1946).

The first experimental evidence that the brain elicits development by stimulating the prothoracic glands (PG) was obtained in experiments performed on *Hyalophora cecropia* (Williams, 1947). A few years earlier Fukuda (1940) had identified the PG as the source of an active molting principle which was subsequently isolated (Butenandt and Karlson, 1954), named "ecdysone" (Karlson, 1956), and ultimately identified as a polyhydroxylated steroid (Huber and Hoppe, 1965). The brain-PG relationship has since been repeatedly documented (Wigglesworth, 1952; Kambysellis and Williams, 1971; Agui, 1975; Bollenbacher, Agui, Granger, and Gilbert, 1979). It forms one of the pillars of current insect endocrinology which views insect development as a progression of stages, each initiated during discrete periods of ecdysone production by the PG under the tropic stimulation of a brain hormone (prothoracicotropic hormone, PTTH).

In point of fact, a role for the brain in insect molting and metamorphosis had detractors even before it had advocates. Beginning with the observations of Conte and Vaney in 1911 and continuing with those of Kopeć himself (1912, 1922), the literature cites multiple examples of metamorphic development by brainless insect preparations at or beyond the late feeding period of larval life (Bounhiol, 1938; Fukuda, 1944; Ozeki, 1954; Ishizaki, 1972; Mala, Granger, and Sehnal, 1977; Meola and Adkisson, 1977). Perhaps no more remarkable observation of development by brainless insects exists than that of Judy (1972) who reported the ability of brainless larvae of the tobacco hornworm, *Manduca sexta,* not only to undergo a larval molt, but also to feed and complete metamorphosis to morphologically normal adults.

Early in our study of the developmental endocrinology of the tobacco hornworm we undertook a reexamination of the role of the brain in this insect's life cycle. An individual of this species in the penultimate instar faces four major developmental transitions before reaching adulthood: a) a larval molt to the fifth instar, b) the feeding-to-wandering transition at the conclusion of the fifth instar, c) the larval-pupal molt, and d) the pupal-adult molt. These steps share several common features. The beginning of each is accompanied by a detectable rise in the hemolymph ecdysone titer (Bollenbacher and Gilbert, unpublished observations cited in Riddiford and Truman, 1978; Bollenbacher, Vedeckis, and Gilbert, 1975; Kaplanis, Thompson, Yamamoto, Robbins, and Louloudes, 1966). Iso-

lated abdomens in all the larval stages are unable to develop (Truman, 1972; Fain and Riddiford ,1976; Truman and Riddiford, 1974; Nijhout, 1976), but such preparations can be induced to molt or metamorphose by injections of ecdysone (Fain and Riddiford, 1976; Nijhout, 1976) or by implantation of active prothoracic glands (personal observations). Moreover, diapausing pupae can be caused to develop by injection of ecdysone, as can isolated pupal abdomens. These observations strongly suggest that each of the four transitions is dependent on ecdysone production by the PG—a conclusion consistent with the classical model of insect development. Yet the aforementioned work by Judy (1972) as well as the observations of Truman and Riddiford (1974), Nijhout (1976), and Wilson and Larsen (1974) suggest that some of these transitions may be partially or completely independent of the brain. In the experiments reported here we have re-examined the role of the brain in each of the four ecdysone-dependent transformations.

Materials and Methods

Larvae were reared at 25° C on an artificial diet, as described by Bell and Joachim (1976) and Truman (1972), under either short-day (SD, 12L: 12D) or long-day (LD, 17L: 7D) photoperiods. Time-of-day was arbitrarily referenced to lights-off at midnight (24: 00 = 00: 00). Larvae of the following types were segregated early in the photophase of each day: freshly ecdysed fourth-instar larvae and pharate fourths showing slipped head capsules, freshly ecdysed fifths, and fifths showing freshly exposed dorsal vessels. The timing of events in the life cycle was as described by Truman (1972) and Truman and Riddiford (1974) except that the first 24 hr of each stage was termed Day 1 rather than Day 0.

Neck-ligatures were placed between the head and prothorax of larvae and between the head and prothoracic spiracles of pupae within 6 hr after pupal ecdysis. Isolated abdomens of larvae were obtained by placing ligatures across the first abdominal segment or between the metathorax and first abdominal segment. In all cases the portion anterior to the ligature was excised. Ligatures were of cotton-covered polyester (J. & P. Coats). Individuals were anesthetized with CO_2 prior to ligation and the surgical procedures.

Operations other than ligation were carried out on individuals immersed in insect Ringer's solution (Ephrussi and Beadle, 1936). Manipulations of the larval brain were performed through a small flap cut to one side of the midline just above the juncture of the cuticular sutures of the head capsule. The brain was easily located and gently drawn toward the incision by the entering bundles of trachea and nerves. These bundles were severed with micro-scissors and the brain withdrawn. Pupal brains were removed through a horizontal incision on the vertex of the head. Loose brains were completely withdrawn from the insect before reimplantation into a lateral recess of the head. Brain implantations into ligatured insects were either by way of a small incision in the dorsolateral aspect of the thorax or abdomen or through an incision in an abdominal proleg which was then closed by a ligature proximal to the incision. Implantations into pupae were through a small incision in the dorsum of the head. All surgical incisions except those in larval prolegs were sealed by apposition of the cut edges, thorough blotting of the region surrounding the incision, and application of a small drop of melted Tackiwax (Cenco). Less than 5% of individuals failed to survive the operations, and these typically died within 1 to 3 days. They have been excluded from the data presented.

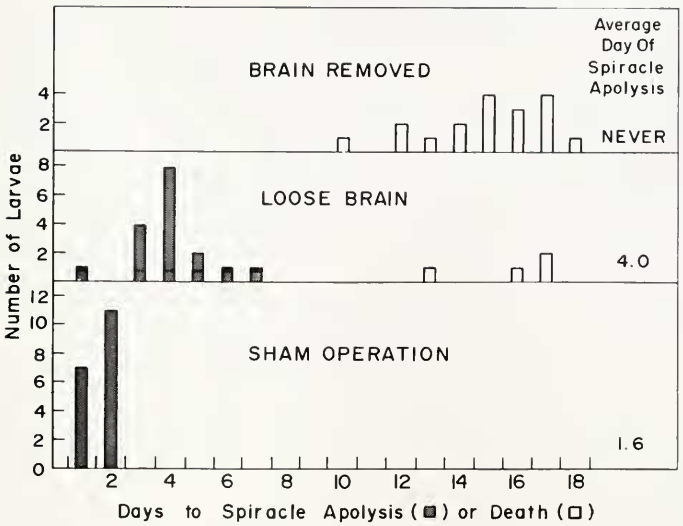

FIGURE 1. Inhibition of molting in fourth-instar larvae by operations involving the brain. Second-gate SD larvae were utilized at 20:00 to 23:00 on Day 3 of the instar. They were afterwards placed without food in individual plastic containers and examined daily for spiracle apolysis (solid box) or death (clear box). Unoperated larvae would typically have undergone spiracle apolysis on Day 1.

Two batches of partially purified PTTH from *Bombyx mori* (Professor H. Ishizaki of Nagoya University, Japan) were tested: one, "Crude PTTH," the second, "Highly-purified PTTH." The lyophilized extracts were dissolved for injection in 0.2 M ammonium acetate as suggested by Prof. Ishizaki. The extracts were tested within 1 week after being dissolved and were injected with a Hamilton syringe equipped with a 30 gauge needle. The biological activity of these extracts was ascertained by injection of an appropriate dose into brainless *Samia ricini* pupae.

The juvenile hormone analog (JHA) Hydroprene (ZR-512, Zoecon Corp.) was used. The compound was of technical grade and was dissolved in reagent grade acetone (Fisher) for topical application.

RESULTS

Molt of fourth-instar larvae

To analyze the role of the brain in larval molting we performed a series of operations on fourth-instar larvae. Second-gate SD larvae were segregated as previously described (Truman, 1972). All operations were performed at 20:00 to 23:00 on Day 3, following which the larvae were returned to individual plastic containers without food.

The results (Fig. 1) show that the brain is indispensable for the molt from the fourth to the fifth instar. Brainless larvae neither molted nor showed any other developmental response although they typically survived for 1.5 to 2.5 weeks. In additional experiments performed on dozens of individuals, no second-gate larva deprived of its brain prior to the third night of the instar ever underwent any further development.

When the brain was removed and then reimplanted into the head, the ability to undertake a larval molt was restored to many but not all individuals after a delay of several days. Autopsy of the larvae completing the molt failed to show reestablishment of any nerve connections except in two individuals where connections appeared to have been reestablished with the subesophageal ganglion or a corpus cardiacum.

When abdomens were isolated from fourth-instar larvae during the above-mentioned 3-hr period, no development took place in any of over 50 preparations. So also, no development was observed in 14 such abdomens receiving brain implants from larvae of the same age.

In another set of experiments, brains were removed from 51 second-gate larvae approximately 12 hr later than in the previous set of experiments—i.e., approximately 10 hr prior to the anticipated apolysis of the head capsule. All individuals underwent a larval molt. The majority experienced considerable difficulty in freeing their heads from the old head capsules; even when assisted in doing so, the head usually was sufficiently deformed in the area of the mandibles as to prevent feeding. Nevertheless a total of 11 of these brainless larvae underwent normal ecdysis and resumed feeding after they were restored to diet. Six of these pupated successfully after a variable delay and of these all ultimately completed adult development as previously described by Judy (1972).

Feeding-wandering transformation of fifth-instar larvae

When second-gate larvae were neck-ligated at times beginning shortly after 00:00 on Day 5, the normal onset of the wandering stage on Day 6, signaled by exposure of the dorsal vessel, occurred on schedule or with little delay. If the brain played a role in the transformation of these larvae into wanderers, the critical period for its action must have been virtually concluded by early on Day 5. For this reason two groups of fifth-instar larvae prior to Day 5 were utilized in a series of operations examining the brain's role at the feeding-to-wandering transition. One group contained second-gate LD larvae weighing 6.5 to 8.5 g and was operated on between 19:00 and 22:00 on Day 4 of the instar. The other group consisted of 4.0 to 4.5 g LD larvae and was operated on early on Day 3 of the instar. All larvae were returned to plastic containers without food following the operation.

The results (Fig. 2) show that the absence of a brain caused a substantial delay in the time to dorsal vessel exposure, this effect being especially pronounced in the smaller individuals. Indeed, many of the larvae in the latter group never exposed the dorsal vessel although they survived for over 3 weeks. Reimplantation of a brain into brainless larvae lessened but did not fully eliminate the delay occasioned by brain removal.

When abdomens were isolated from larvae similar to either of the above groups, no development took place during the 2 to 3 weeks of survival. Nor was development provoked in any of 16 abdomens isolated from larvae of the older class when a brain from individuals of the same age was implanted.

Wandering larva-pupa transformation

A series of operations was performed on 60 SD wandering larvae at 12:00 to 15:00 on the day of dorsal vessel exposure. Larvae were examined daily thereafter for pupation. Unoperated larvae pupated after 5.2 ± 0.4 days, sham-

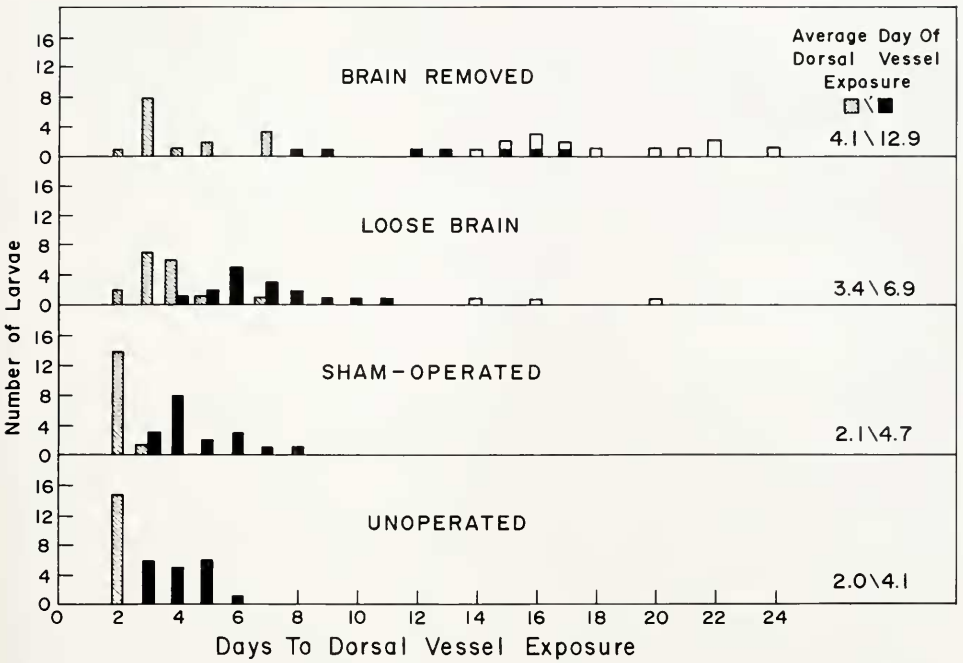

FIGURE 2. Inhibition of dorsal vessel exposure in fifth-instar larvae by operations involving the brain. LD larvae in two weight classes were utilized: 6.5 to 8.5 g larvae (hatched box) and 4.0 to 4.5 g larvae (non-hatched boxes). Larvae of the smaller class which died prior to dorsal vessel exposure are designated by clear boxes, those which exposed the dorsal vessel by solid boxes. Following operation, the larvae were returned to individual plastic containers without food and examined daily thereafter for dorsal vessel exposure or death. Averages were computed only for those larvae which exposed the dorsal vessel.

operated larvae after 6.0 ± 0.0 days, and brainless pupae after 7.2 ± 0.8 days. Wandering larvae lacking a brain were thus unquestionably delayed relative to both unoperated and sham-operated controls. As with the younger larvae, abdomens isolated from wandering larvae were unable to pupate. And when brains from Day 1 wandering larvae were implanted into 11 such abdomens, no development occurred during the subsequent 1.5 to 3 weeks of survival.

Pupal-adult transformation

To examine the role of the brain in the regulation of adult development and of pupal diapause, a series of operations was performed on LD and SD pupae during the 12 hr following pupal ecdysis. The results (Fig. 3) demonstrate the ability of the brain to provoke the initiation of adult development as signaled by apolysis of the wing epidermis. Both LD and SD pupae deprived of their brains experienced a substantial delay before initiating development—far beyond that observed in intact, diapausing pupae. The reimplantation of loose brains greatly curtailed but did not fully eliminate this delay. Loose SD brains stimulated development only after a latent period similar to that encountered in normal diapausing pupae; this was so irrespective of whether the host was a SD pupa or a brainless LD pupa. Loose LD brains uniformly stimulated prompt development and appeared to function with identical effect in either LD or SD hosts.

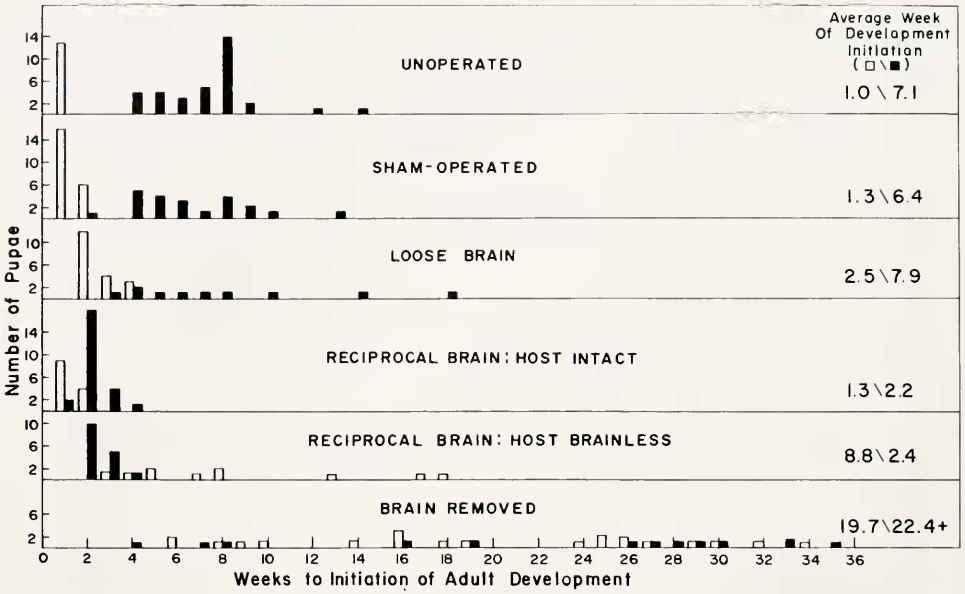

FIGURE 3. Initiation of adult development following operations involving the pupal brain. Diapause-destined SD pupae (solid box) and non-diapausing LD pupae (clear box) were utilized within 12 hr of pupation and examined weekly thereafter for initiation of adult development as signaled by apolysis of the wing epidermis. Sham-operated pupae were incised and the brain visualized but not touched. In the case of reciprocal brain implants, a brain from a pupa of the alternate photoperiod was implanted into a normal or brainless pupa; in the figure the graphic designation indicates the diapause program of the pupal host. In the case of brainless SD pupae, 4 individuals had not yet initiated development at 36 weeks.

In all cases development once initiated proceeded to completion in the usual period of about 18 days.

Identity of the brain tropic factor throughout the life cycle

We tested the ability of larval and pupal brains to cause the molting of larvae and the adult development of pupae. The larval brains were obtained from LD second-gate fifth-instar larvae between 07:00 and 12:00 on Day 5; the pupal brains from LD animals 6 to 12 hr after pupal ecdysis. These were implanted into diapausing pupae and into second-gate fourth-instar larvae head-ligated prior to 21:00 on Day 3 of the instar; the latter were topically treated with 20 μg of JHA to permit larval molting. The host pupae would normally have undergone development after an average of 1 to 2 months, while the larval hosts would normally have undergone no development whatsoever. Development of the host was recognized by initiation of apolysis in pupae and by spiracular apolysis in larvae. The results of these experiments were unambiguous (Table I). Larval as well as pupal brains caused the molting of larvae and the adult development of pupae.

Localization of prothoracicotropic activity in the nervous system

Several ganglia from Day 5 second-gate fifth-instar larvae were examined for their ability to elicit the development of diapausing pupae: these included the

TABLE 1

Effects of implanted larval and pupal brains on the development of larval and pupal hosts.

Donor	Host	Number of assays	Percent developing	Time to initiation of development (days)
Larva	Pupa	12	100	10
Pupa	Pupa	15	100	9
Larva	Larva	10	80	4*
Pupa	Larva	13	69	5*

* Averages computed only for developing individuals.

brain, the subesophageal, prothoracic, mesothoracic, and terminal abdominal ganglia. Epidermis was implanted as a control. Single implants were made into diapause-destined pupae 6 to 18 hr after pupal ecdysis. Each type of ganglion was assayed in 15 to 17 pupae, except for the mesothoracic ganglion, which was assayed in 8 pupae. Activity was measured by averaging the time to initiation of development by the assay pupae, determined by apolysis of the wing epidermis. Only the brain possessed any significant tropic activity. Pupae receiving brains initiated development an average of 1.5 weeks later. Groups of assay pupae receiving other ganglia took from 5 to 7 weeks to initiate development and did not differ significantly from pupae receiving an implant of epidermis, which took an average of 6 weeks.

Assay of Bombyx mori *PTTH extracts*

We attempted to demonstrate a prothoracicotropic effect of *Bombyx mori* PTTH extracts in larvae and pupae of *Manduca sexta*. For assay of this activity we used 3-week old diapausing pupae, 1-week old diapausing pupae whose brains had been removed on the day following ecdysis, and fourth-instar larvae whose brains had been removed prior to PTTH release. Both crude and highly-purified PTTH preparations were inert in each of these bioassays at concentrations up to 100 *Samia* units, the highest dose tested.

DISCUSSION

Brain removal significantly delayed the development of larvae and pupae when carried out at appropriate intervals prior to the initiation of ecdysone-mediated transformations. The molting of larvae and the onset of adult development in pupae were especially delayed. When loose brains were reimplanted into the head, the delay occasioned by brain removal was consistently reduced but not eliminated. Abdomens isolated from larvae at various stages never undertook development nor were they caused to develop by the implantation of active brains. These results are consistent with the classical view of the brain as the source of a hormone which stimulates ecdysone production by the prothoracic glands at each ecdysone-dependent transition.

Judy (1972) noted a larval-larval molt in only one exceptional individual among his many brainless fourth-instar larvae. The present results (Fig. 1) further document the absolute necessity of the brain for larval molting. Brainless fourth-instar larvae resumed development only after the brain had played its role or after restoration of an active brain.

In the case of pupae, both diapausing and non-diapausing individuals required a brain in order to initiate adult development on schedule. Removal of the pupal brain routinely delayed the initiation of development by weeks or months, while implantation of loose brains restored the development of brainless hosts to a nearly normal rate. Pupal brains demonstrated the remarkable ability to retain their original commitment to diapause or not to diapause even when detached from all connections. Thus, as shown in Figure 3, brains from non-diapause pupae triggered prompt initiation of development, whereas brains from diapause-destined pupae caused development only after a delay typical of such brains *in situ*. Retention of normal endocrine behavior by loose insect brains has been previously observed (Williams, 1946; Williams and Adkisson, 1964; Truman and Riddiford, 1970).

Remarkable, too, was the failure of the host environment to affect the diapause or non-diapause programs of implanted pupal brains. Whenever the host received a brain from a non-diapausing donor, the host initiated development promptly regardless of its own program; in turn, brains from diapause-destined pupae caused development of brainless LD hosts only after a delay whose duration was similar to that of a normal diapause. When diapause-destined brains were implanted into intact non-diapausing hosts, they did not deter the prompt development stimulated by the host's brain. Hence, the timetable for development appears to be programmed into the pupal brain. These data suggest that *Manduca* possesses a classical pupal diapause in that the developmental standstill is due to a temporary cessation of the brain's tropic action on the PG.

The sum total of the present evidence clearly indicates that the brain normally exercises a prothoracicotropic function at each ecdysone-mediated transition in the tobacco hornworm. But while removal of the brain prevented or greatly delayed the development of fourth-instar larvae and of pupae, its effects where much less dramatic in feeding or wandering fifth-instar larvae. Furthermore, the eventual development of brainless *Manduca* pupae contrasts with the classical examples of complete developmental arrest following brain removal in saturniids (Williams, 1946).

Exceptions to the current model of insect development can be grouped as contradictions to one or more of four assumptions implicit in the classical scheme: a) Only the PG produce ecdysone; b) Only the brain activates the PG; c) PG function is entirely dependent on stimulation by the brain and, consequently, fluctuations in PG activity reflect changes in the output of the brain's PTTH; d) The onset of development occurs only after a particular quantity of ecdysone has been secreted.

Though the production of ecdysone by the PG is no longer disputed (Chino, Sakurai, Ohtaki, Ikikawa, Miyizaki, Ishibashi, and Abuki, 1974; King and Marks, 1974; King, Bollenbacher, Borst, Vedeckis, O'Connor, Ittycheriah, and Gilbert, 1974; Romer, Emmerich, and Novock, 1974), other sources of ecdysone are obviously present in at least certain species. For example, ovarian synthesis of ecdysone has been documented in several species (Hagedorn, O'Connor, Fuchs, Sage, Schlaeger, and Bohn, 1975; Lagueux, Hirn, and Hoffman, 1977; Bollenbacher, Zvenko, Kumaran, and Gilbert, 1978). Moreover, ecdysone production and even ecdysone-dependent development have been observed in isolated abdomens and preparations lacking prothoracic glands (Chadwick, 1955; Nakanishi, Moriyama, Okauchi, Fujioka, and Koreeda, 1972; Hsiao, Hsiao, and DeWilde, 1975; Studinger and Willig, 1975; Gersch and Eibisch, 1977; Delbecque, Delchambre,

Hirn, and DeReggi, 1978). The role of these other ecdysone sources during normal molting and metamorphosis remains an enigma. Nevertheless, the failure of isolated abdomens of tobacco hornworm larvae to undertake any development even when supplied with an active brain suggests that sources of ecdysone other than the PG are unlikely to account for the development of brainless individuals in the final larval stage of this species.

While the brain is certainly the chief source of tropic input to the PG, the literature documents several potential mechanisms for PG activation in the absence of the brain. Injury has been demonstrated to stimulate pupal development (McDaniel and Berry, 1967; Wilson and Larsen, 1974), but in the present study sham operations to larvae or freshly ecdysed *Manduca* pupae tended to delay rather than accelerate development. PTTH activity has been identified outside the brain of some insects in the corpora cardiaca, the corpora allata, or elsewhere (Ichikawa and Nishiitsutsuji-Uwo 1959; Gersch and Stürzebecher, 1968; Ishizaki, 1969; Gibbs and Riddiford, 1977). Moreover, the corpora allata could potentially stimulate ecdysone production by virtue of their ability to produce juvenile hormone (Williams, 1959; Gilbert and Schneiderman, 1959; Hiruma, Shimada, and Yagi 1978). Nevertheless, in the present study bioassays of several ganglia failed to disclose significant PTTH activity in the nervous system outside the brain of *Manduca sexta*—a result in keeping with the findings of Gibbs and Riddiford (1977). Moreover, larvae and pupae lacking both brains and CA can undergo metamorphic development (Judy, 1972; Truman and Riddiford, 1974; personal observations).

Bombyx PTTH failed to elicit development of appropriate assay animals in the present study even though doses up to 100 times the amount necessary to wake up brainless *S. ricini* pupae were employed. Similar interspecific failures have been noted previously in studies using intact brains as well as brain homogenates as PTTH sources, although other such attempts have met with success (Gibbs and Riddiford, 1977; Williams, 1946; Ichikawa and Nishiitsutsuji, 1951). Assay of active brains from *Manduca* larvae or pupae demonstrated that both could accelerate ecdysone-dependent development in larval as well as pupal preparations (Table I). These data agree with prior results: for *Manduca* by Gibbs and Riddiford (1977), for *H. cecropia* by Williams (1952), and for *B. mori* by Kobayashi, Yamazaki, and Kimura (1973). Though the detailed chemistry of PTTH may vary between species, no convincing evidence supports the existence of more than a single native form within a particular species.

In the absence of a significant prothoracicotropic factor outside the hornworm brain, the PG might still secrete varying amounts of ecdysone if in the course of metamorphosis they exhibited stage-specific rates of spontaneous ecdysone production. Alternatively the PG might be subjected to stage-specific levels of inhibition. Hinton (1953) suggested that the PG might be inhibited as well as stimulated, but little clear evidence presently supports this possibility. An inhibitory factor has been supposed to originate variously from the brain (Carlisle and Ellis, 1968), from the subesophageal ganglion (Alexander, 1970), or from the thoracic ganglia (Mala, Granger, and Sehnal, 1977). The evidence is at best fragmentary and at times contradictory. Nevertheless the existence of a PG inhibitor arising outside the brain and variously employed during the life cycle remains an attractive explanation for the different rates of development by brainless individuals in the present experiments.

While molting and metamorphosis unquestionably require exposure to par-

ticular amounts of ecdysone, the relationship between the quantity of ecdysone necessary for a developmental response and the quantity produced is by no means a direct one. To trigger a developmental response the requisite amount of ecdysone must be produced, appropriately metabolized, and accumulated to effective intracellular concentrations. Furthermore, the visible start of development may be preceded by so-called "covert effects" (Ohtaki, Milkman, and Williams, 1968). Breakdown of ecdysone counters production, and even the covert effects of ecdysone may undergo decay (Ohtaki, Milkman, and Williams, 1968; Zdarek and Fraenkel, 1970). A small change in any one of these variables could stagger the rates of development of two individuals with identical rates of ecdysone production. Such a difference could well account for the contrasting behaviors of brainless cecropia pupae, which never develop (Williams, 1946), and brainless hornworm pupae, which finally develop after latent periods varying from weeks to months. Manifestly, the same considerations could influence the rates of development of brainless specimens of *Manduca* in the several developmental stages.

Removal of the brain unmasks aspects of ecdysone secretion, metabolism, and action which are normally hidden by the dominant rate-determining activity of PTTH. We have considered four assumptions underlying the classical model, one or more of which must be violated for brainless individuals to develop. The present data suggest that in *Manduca* only the PG produce ecdysone in amounts and at periods relevant to molting and metamorphosis. Moreover, the results here cannot readily be accounted for by the existence of prothoracicotropic factors originating outside the brain. The proper explanation of our findings therefore presumably lies in some combination of the following: changes in the autonomous activity of the PG; changes in the degree of inhibition of the PG; changes in ecdysone metabolism; changes in the sensitivity of the epidermis to ecdysone. As we shall demonstrate subsequently, several of these mechanisms seem in fact to have an important bearing on the development of the tobacco hornworm. But as the story unfolds, the reader must not forget that in every stage of development the brain plays a front and center role. While the present investigation suggests the possible existence of additional mechanisms of regulation, its results on the whole pay tribute to the brain of Stefan Kopeć.

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Summary

1. Brain removal at appropriate times in the life cycle uniformly delayed or prevented the onset of all known ecdysone-mediated transitions. This effect was especially pronounced at larval-larval molts and at the pupal-adult transformation.

2. Loose brains reduced but did not eliminate the delay induced by brain removal.

3. Isolated larval abdomens never developed nor were they induced to do so by implantation of an active brain.

4. Loose pupal brains retained their original commitment to diapause or not

to diapause. This was true even when the loose brain was implanted into a brainless host pupa with an opposite diapause commitment.

5. Larval brains effectively elicited the development of pupae, and conversely, pupal brains elicited the development of larvae.

6. Prothoracicotropic activity was found in the brain but in no other ganglia.

7. Extracts of *Bombyx mori* PTTH were inactive in *Manduca* in the concentrations tested.

8. Consideration is given to several mechanisms which might mediate the development of brainless hornworms.

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